PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Impact of carbohydrate reduced nutrition in septic patients on ICU - study protocol for a prospective randomized controlled trial
AUTHORS	Rahmel, Tim; Hübner, Max; Koos, Björn; Wolf, Alexander; Willemsen, Katrin-Maria; Strauß, Gabriele; Effinger, David; Adamzik, Michael; Kreth, Simone

VERSION 1 – REVIEW

REVIEWER	Federico Bozzetti Faculty of Medicine, University of Milan
REVIEW RETURNED	19-Mar-2020

GENERAL COMMENTS	I am somewhat concerned about this study because it seems to me that much emphasis is put on the potential deleterious effects of glucose while neglecting the fact that bone marrow, CNS and granulation tissue rely on glucose metabolism. However my main criticism regards the objective of the study. If the primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days , why to perform a randomized clnical trial?.

REVIEWER	Henk M. De Feyter Yale University, USA
REVIEW RETURNED	10-Apr-2020

GENERAL COMMENTS	The authors describe a ongoing study looking to evaluate feasibility, efficacy and safety of diet-induced ketosis in ICU patients with sepsis. The rationale of the study is clearly presented. This study will provide useful novel information on the applicability of diet-induced ketosis, in a novel target population. Given that the authors are motivated to publish their study protocol, does it make sense to also register on ClinicalTrials.gov? p.4, line 50: the authors refer to another paper and mention a reduction of carbohydrates to 10%. Suggest to clarify 10% of what? p. 7. line 37: effect size is discussed, which parameter does effect size apply to? BHB levels in blood? p.9 line 40: there is no mention of controlling or measuring calorie intake. As with every study focused on dietary intervention, this could be a critical component. Please provide more details and/or discuss.

	p.10, line 12: which ketone bodies will be measured (concentration) in blood and urine? All 3 of them? Question, can be addressed in discussion: would the study benefit from a target ketone body level? If the BHB levels increase with 20%, and it turns out to be statistically significant, it's still a low level of BHB. Additionally, would it be relatively easy to increase plasma ketone body levels by IV in this population?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:
1. I am somewhat concerned about this study because it seems to me that much emphasis is put on the potential deleterious effects of glucose while neglecting the fact that bone marrow, CNS and granulation tissue rely on glucose metabolism. However, my main criticism regards the objective of the study.
Response: Thank you very much for this valuable comment. Current nutrition recommendations in critical ill are providing amounts of glucose beyond minimal needs and thus preventing ketosis. However, the need to provide amounts of glucose above minimal needs is exactly what has never been demonstrated. Furthermore, during a low-carb diet the regulated and controlled production of ketone bodies is known to cause a harmless (potentially even favourable) "substitute" physiological state known as dietary ketosis.1 In this situation, ketone bodies are provided from the liver to extra-hepatic tissues (e.g. CNS and bone marrow) as alternative energetic supply. This spares glucose metabolism via utilisation of ketone bodies that can also be metabolized in the CNS. Moreover, blood glucose levels remain within the physiological range under low-carb nutrition due to glucogenic sources (glucogenic amino acids and lipolysis-derived glycerol) that are still provided in ketogenic diets.2 However, this clinical trial aims at providing first solid evidence regarding safety and feasibility (as important outcome measures) of a ketogenic diet in septic patients. To discuss this issue more precisely we applied the following changes to our revised manuscript.
Changes made in the manuscript:
- Discussion, page 14-15, line 23(14)-8(15)
Currently, state-of-the-art nutrition in critically ill patients contain more than 40% carbohydrates, thus exceeding minimal needs and preventing ketosis. However, the need to provide amounts of glucose above minimal needs in these patients has never been demonstrated. Furthermore, during a low-carb diet in healthy adults the controlled production of ketone bodies is known to cause a harmless (and potentially even favourable) "substitute" physiological state known as dietary ketosis. In this situation, ketone bodies are provided from the liver to extra-hepatic tissues (e.g. CNS) as alternative energetic supply. This spares glucose metabolism via utilisation of ketone bodies as an alternative fuel. Moreover, blood glucose levels remain within the physiological range under low-carb nutrition due to glucogenic sources (glucogenic amino acids and lipolysis-derived glycerol) that are still provided in ketogenic diets.
2. If the primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days , why to perform a randomized clinical trial?
Response: As mentioned above, our study aims at assessing the safety and feasibility of a ketogenic / low-carb diet. Thus, a control group as well as the randomizedcontrolled trial design is of utmost importance to provide solid evidence for

causality. Accordingly, we added this information as new bullet in the strength and limitation section of the revised manuscript.
Changes made in the manuscript:
- Strengths and limitations of this study, page 3, line 10-12:
Our controlled and longitudinal study design will allow us to interpret alterations over time in the intervention and control group, and will provide strong evidence for causality.
Again, thank for your time, helpful comments, and effort spent in improving our manuscript.
References
1. Feinman RD, Makowske M. Metabolic syndrome and low-carbohydrate ketogenic diets in the medical school biochemistry curriculum. Metab Syndr Relat Disord 2003;1:189-97.
2. Veldhorst MA, Westerterp-Plantenga MS, Westerterp KR. Gluconeogenesis and energy expenditure
after a high-protein, carbohydrate-free diet. Am J Clin Nutr 2009;90:519-26.

Reviewer 2:
1. Given that the authors are motivated to publish their study protocol, does it make sense to also register on ClinicalTrials.gov?
Response: Thank you for this valuable comment. As mentioned in our manuscript, the study is already registered in the German trail register (www.DRKS.de) under the identifier DRKS00017710. The DRKS is an approved Primary Register (equivalent to ClinicalTrials.gov) in the WHO network since October 2008 and thus meets all requirements of the ICMJE. Therefore, we assume that an additional registration in ClinicalTrials.gov is not necessary. However, if you still recommend an additional registration as mandatory, we will follow your advice accordingly.
2. p.4, line 50: the authors refer to another paper and mention a reduction of carbohydrates to 10%. Suggest to clarify 10% of what?
Response: During this diet, 10% of the overall calorie intake should be administered as carbohydrates. This is now clarified within the manuscript.
Changes made in the manuscript:
- Introduction, page 4, line 21:
In these studies, the total amount of carbohydrates is reduced to approximately 10% of the overall calorie intake, whereas protein amounts are kept constant and fat amounts are increased.
3. p. 7. line 37: effect size is discussed; which parameter does effect size apply to? BHB levels in blood?
Response: Thanks for pointing out this issue. The mentioned effect size estimation refers to β-hydroxybutyric acid concentrations in blood. We have now added this information within the revised manuscript.
Changes made in the manuscript:
- Methods and analysis, page 7, line 13-17:
Based on available data on ketogenic diet regimes for healthy individuals referring to the β-hydroxybutyric acid blood concentration11 and our estimation of a clinical reasonable effect size, we assume an effect size (Cohen's d) between 1.34 and 2.14 as appropriate.
4. p.9 line 40: there is no mention of controlling or measuring calorie intake. As with every study focused on dietary intervention, this could be a critical component. Please provide more details and/or discuss.
Response: Thank you very much for addressing this central aspect in our study. In response to your comment, we now have implemented this information in our revised manuscript.
Changes made in the manuscript:
- Methods and analysis, page 8-9, line 22(8)-4(9):

The energy expenditure to determine the daily calorie goal is estimated by using indirect calorimetry (Q-NRG+, COSMED, Rome, Italy). The enteral nutrition is commenced at an initial rate of 20 mL/h, and increased by 20 mL/h every 6 h in the absence of significant gastric residuals (i.e., ≥ 500 mL), with the aim of reaching the estimated calorie goal within 24 h after study enrolment. The attending physician is responsible for ensuring the achievement of energy targets. The exact calorie intake is electronically recorded and saved in the electronic health records.

5. p.10, line 12: which ketone bodies will be measured (concentration) in blood and urine? All 3 of them?

Response: As primary endpoint we will quantify hydroxybutyric acid concentrations in blood. This is now stated in the manuscript,

Changes made in the manuscript:

- Methods and analysis, page 8-9, line 22(8)-4(9):

The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase hydroxybutyric acid concentration in blood within 14 days.

6. Question, can be addressed in discussion: would the study benefit from a target ketone body level? If the BHB levels increase with 20%, and it turns out to be statistically significant, it's still a low level of BHB. Additionally, would it be relatively easy to increase plasma ketone body levels by IV in this population?

Response: Thank you for this interesting question. To date, there is no data addressing these questions for septic patients. Accordingly, it is difficult to draw specific assumptions. Our experience so far gained in healthy volunteers does not suggest an underlying dose effect (in terms of the higher the better). We could rather see that the favourable effects of ketones are associated with the metabolic switch to ketosis (increase of BHB approximately above levels of 0,5-0,8 mM). The absolute concentration seems to be less decisive.

Therefore, we do not expect that the substitution of ketone bodies(albeit it being a sophisticated idea) is able to mimic the effects of a low-carb nutrition due to the absence the metabolic switch. In addition, the substitution of ketone bodies is only rudimentarily investigated and merely applied strategy, but also with serious inherent limitations (such as salt overload).

However, at this moment, these considerations are only of a speculative nature. We are convinced that our work will provide useful novel information concerning this point. Therefore, we would appreciate taking up these interesting questions and discuss them along with our upcoming results expected at the end of next year.

Again, thank for your time, helpful comments, and great effort spent in improving our manuscript.

VERSION 2 – REVIEW

REVIEWER	Federico Bozzetti ASLC
REVIEW RETURNED	22-Apr-2020

GENERAL COMMENTS	1. It is clear that, despite the opposite opinion and answer of the authors, this is a biologic, not a clinical study, aiming to demonstrate whether a KD may increase the BHB level in the blood. There is no need of having a randomised control group. 2. If the authors believe that increasing the BHB level in the blood is useful for their patients and the first step to validate this hypothesis is increasing the BHB level in the blood, the simple way is to

	administer KB by mouth. For instance, giving H.V.M.N. Ketone Ester orally in controlled dosages, can produce plasma KB levels comparable to those achieved by the most rigorous KD, while avoiding the potential consequences of glucose deprivation to glucose-dependent tissues (1). 3. Finally the authors do not appear having properly considered the occurrence of some side-effects of the KD (2). Ref. 1 Hashim SA, VanItallie TB. Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester. J Lipid Res. 2014;55(9):1818–1826. doi:10.1194/jlr.R046599 2 Bostock EC, Kirkby KC, Garry MI, Taylor BV. Comparison of precipitating factors for mania and partial seizures: Indicative of shared pathophysiology?. J Affect Disord. 2015;183:57–67. doi:10.1016/j.jad.2015.04.057

REVIEWER	Henk M. De Feyter Yale University, USA
REVIEW RETURNED	26-Apr-2020

GENERAL COMMENTS	Thank you for addressing my concerns and questions.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1:

1. It is clear that, despite the opposite opinion and answer of the authors, this is a biologic, not a clinical study, aiming to demonstrate whether a KD may increase the BHB level in the blood. There is no need of having a randomised control group.

Response: We have again discussed your comment with the authors and other experts. We also rechecked our study design with an official and world-wide accepted definition of “clinical studies” and “clinical trials” as described by “clinicaltrials.gov” (https://clinicaltrials.gov/ct2/about-studies/learn).

a. “A clinical study involves research using human volunteers (also called participants) that is intended to add medical knowledge.”

We believe beyond any doubt that critical ill patients suffering from sepsis are sufficient to meet this definition.

b. “In a clinical trial, participants receive specific interventions according to the research plan or protocol created by the investigators.”

Our intervention is the ketogenic diet, which is examined against a "conventional"

nutritional concept (control). Hence, we see the compatibility of our study with this definition, especially since the detection of ketone bodies in the blood just formally verifies the successful induction of ketosis (as causal effect of our intervention) in septic patients along with different safety and outcome measures.

After these explanations, we continue to consider it acceptable to call our study a clinical trial. Furthermore, we disagree that our study does not benefit from a randomized control group. Instead we only see potential merits by implementing a randomized control group, e.g. by reducing potential biases and limitations in data interpretation, without any significant cons.

2. If the authors believe that increasing the BHB level in the blood is useful for their patients and the first step to validate this hypothesis is increasing the BHB level in the blood, the simple way is to administer KB by mouth. For instance, giving H.V.M.N. Ketone Ester orally in controlled dosages, can produce plasma KB levels comparable to those achieved by the most rigorous KD, while avoiding the potential consequences of glucose deprivation to glucose-dependent tissues (1).

Response: To date, there is no data addressing the interesting question, whether the increase in BHB alone is the only important factor that confers beneficial effects of low-carb nutrition. We even expect that favorable effects of low-carb nutrition are additionally associated with the metabolic switch to ketosis. This is corroborated by our preliminary data in healthy volunteers not suggesting an clear underlying dose effect (in terms of the higher the better). Therefore, we assume that the substitution of ketone bodies is not capable to mimic all effects of a low-carb nutrition e.g. due to the absence the metabolic switch. In addition, the substitution of ketone bodies (as salt and esters) is only rudimentarily investigated, but also associated with serious inherent limitations (such as salt overload, pH alterations or gastrointestinal side-effects).

Nevertheless, we agree that ketone bodies might also represent a suitable approach, but this is a completely different intervention and beyond the scope of the current study. However, to adequately discuss this alternative approach we

now have included a corresponding comment in our discussion.

Moreover, significant glucose deprivation in glucose dependent tissues does in our opinion not necessarily represent an inevitable problem, because glucose levels remain within the physiological range, as already clearly stated in our manuscript (page 15, lines 6-8). Furthermore, during sepsis hyperglycemia and insulin resistance are more common problems making glucose deprivation rather subordinate.1 Therefore, we added an corresponding comment in our discussion.

Changes made in the manuscript:

- Discussion, page 15, line 16-19

An alternative way that likewise could confer the beneficial effects of ketone bodies is the direct supplementation of ketone esters and salts.2 However it is not clear if the substitution of ketone bodies is capable to mimic all effects of a low-carb nutrition e.g. due to the absence of the metabolic switch.3

- Discussion, page 15, line 9-10

Furthermore, hyperglycaemia and insulin resistance are more common complications during sepsis suggesting glucose deprivation as subordinate problem.1

3. Finally the authors do not appear having properly considered the occurrence of some sideeffects of the KD (2).

Response: We completely agree that in particular safety issues should be of highest importance in a clinical trial. Actually, we paid a lot of attention to the proper selection and standardized assessment of all relevant side effects (as already mentioned and discussed in our manuscript), but of course we are open to further improvements.

The suggested work of Bostock and colleagues describes different precipitating factors for mania and partial seizures, but unfortunately, does not mention nutrition or ketone bodies at all. Thus, we assume a citation error. Nevertheless, we again checked the current literature regarding missing side effects, but did not find any missing issues.

References:

1. Van Cromphaut SJ, Vanhorebeek I, Van den Berghe G. Glucose metabolism and insulin resistance

in sepsis. Curr Pharm Des 2008;14(19):1887-99. doi: 10.2174/138161208784980563 [published Online First: 2008/08/12]

2. Hashim SA, VanItallie TB. Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester. J Lipid Res 2014;55(9):1818-26. doi: 10.1194/jlr.R046599 [published Online First: 2014/03/07]

3. Marosi K, Moehl K, Navas-Enamorado I, et al. Metabolic and molecular framework for the enhancement of endurance by intermittent food deprivation. FASEB J 2018;32(7):3844-58. doi: 10.1096/fj.201701378RR [published Online First: 2018/02/28]

Reviewer 2:

None. Thank you again for the constructive review process.